On the Labral Glands of a Cladoceran (Simocephalus vetulus), with a description of its mode of feeding.

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With Plates 9, 10 and 2 Text-figures.

Leydig (**12**) in 1860 was the first worker to point out that the possession of labral glands is common to all the Cladocera. In 1846, however, Schödler (**16**) had observed that in the labrum of Acanthocercus there exist paired glandular bodies; he states, 'Im sog. Labrum (des Acanthocercus) glauben wir ein paar rundliche, fast nierenförmige Conglomerate als drüsige Körper (vielleicht als Speicheldrüsen, glandulae salivales) ansprechen zu müssen'. Claus (**3**) in 1876 mentioned these glands in his work on the anatomy of Daphnids, and later Cunnington (**5**) in 1903 described them in Simocephalus sima (Simocephalus vetulus).

Among the other Phyllopoda, Claus (**4**) in 1886 mentions and figures the glands in Branchipus and Artemia. Referring to the labrum he states: 'endlich in dem terminalen Theil die grossen als Speicheldrüsen gedeuteten Drüsenzellen, deren Ausfuhrgangsöffnung und Drüsenstructur auf Querschnitten leicht zu constatieren sind'. Sars (**15**) states that these glands exist in Limnadia and Limnetis, and in his figures of other Phyllopoda large cells are indicated in the interior of the labrum.

With regard to the anatomy Claus (**3**) was the first to give a description in any detail, but apart from this the only description at all complete is due to Cunnington in his description of the glands in Simocephalus sima. Claus considered that

the glands could be separated into two groups, the first group lying under the brain and over the oesophagus and the second group consisting of very large cells lying nearer the tip of the labrum. The first group sent out a long thin efferent duct which, after making many twists, allowed the exit of the secretion in front of the mouth. Cunnington's description differs essentially from this in that he could not observe a duct from the first group but did observe an efferent duct from the second group. Cunnington also distinguishes two groups of cells—a proximal group of several small cells and a distal group of large cells. The proximal group, he states, lie close against the chitinous cuticle and are obviously modified epidermal cells and possibly act as replacement cells, taking the place of cells in the distal group when these lose their secretory power. The latter group usually consists of four cells only and these are placed one behind the other, the most extreme possessing a duct opening on the inner side of the labrum. They have characteristic nuclei, which are shaped like a hollow bowl and thus appear circular or semi-circular in section. The secretion is formed in the neighbourhood of the nuclei in the form of little drops which fuse to larger drops or rods or bands and pass to the exterior. Cunnington suggests that the duct of the extreme cell of the distal group acts as a common duct for the whole group.

Methods.

For Simocephalus vetulus the best fixative was found to be cold saturated sublimate in distilled water. This gave excellent fixation and did not produce distortion as did most other fixatives. Good results were also obtained with a mixture of equal parts of saturated sublimate in distilled water and 1 per cent. osmic acid. This mixture, a modification of Mann's fixative, was allowed to act for about an hour. In comparing Simocephalus with other Cladocera, it was found that for Daphnia the best results were obtained with sublimate acetic acid, while for Graptolebris and Camptocercus Carnoy gave the best fixation. A young Chiro-

cephalus metanauplius was fixed in cold saturated sublimate and was found to be very well fixed.

Ehrlich's haematoxylin was used considerably for staining. Iron haematoxylin gave too intense a stain for the gland-cells. The best differential stain, however, was obtained by using Mallory's triple method for connective tissue.

The fixed material was embedded direct into paraffin and cut 8 μ.

On the Anatomy of the Labral Gland.

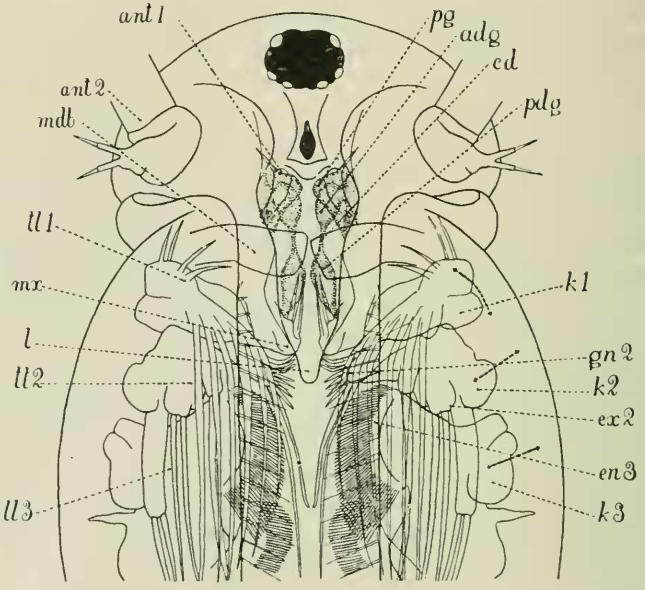
The two groups of gland-cells, as described by Cunnington, were found to be very distinct and will be described separately, but before doing so the extent and position of the labrum must be stated. The labrum, or upper lip, is an immediate prolongation backwards of the ventro-posterior part of the head, passing ventrally to the two laterally-working mandibles and ending under the maxillae which are immediately behind the mandibles. When viewed from the ventral side it may be described as dagger shaped, but its contour is peculiar and reference must be made to Text-fig. 1, which is a ventral view of the animal as it is seen resting normally in a watch-glass, and to Pl. 10, fig. 13 which is a diagrammatic lateral view of the animal. Anteriorly the labrum is marked off from the dorsal part of the head by a groove on each side (Pl. 9, fig. 3) which extends forward to the level of the nauplius eye and then expands dorsally into the bay from which arises the second antenna.

In the living animal the labral glands can be seen indistinctly in the anterior part of the labrum and are of a pale-yellow colour, as was observed by Leydig (12).

Proximal Group.—This consists of two laterally placed groups of epidermal cells which almost meet in the mid-ventral line between the first antennae. Each group commences just in front and close to the ganglion of the nerve to the first antenna (Pl. 10, fig. 13), and extends postero-dorsally over a lozenge-shaped area lining the lateral cuticle of the labrum as far back as the labral nerve (Pls. 9 and 10, figs. 1–4 and 13). Each group consists of about twenty cells, and the nuclei of these

vary in size, being smallest at the base of the first antennae and largest about the centre of the group. The nuclei are usually about 20 μ long, but the smallest are never less than half this

TEXT-FIG. 1.



Semi-diagrammatic ventral view of Simocephalus vetulus. The thick dotted lines ending in arrow heads on the animal's left side indicate the direction and extent of the normal movement of the appendages figured on that side.) *adg*, anterior pair of distal gland-cells; *ant* 1, first antenna; *ant* 2, second antenna; *cd*, connexion between anterior and posterior pairs of distal gland-cells; *en* 3, proximal endite of third trunk-limb; *ex* 2, exopodite of second trunk-limb; *gn* 2, gnatho-base of second trunk-limb; *k* 1, *k* 2, *k* 3, branchiae of first, second, and third trunk-limbs respectively; *l*, labrum; *mdb*, mandible; *mx*, maxilla; *pdg*, posterior pair of distal gland-cells; *pg*, proximal gland; *tl* 1, *tl* 2, *tl* 3, first, second, and third trunk-limbs respectively.

length. For comparison it may be stated that the length of the nuclei of nerve-cells or of muscle-cells, which are of very uniform size and oval shape, is 4 μ. Thus the volume of these large gland-cell nuclei must be many times, at least twenty

times, that of the nucleus of a nerve- or muscle-cell. The chromatin in these nuclei is distributed fairly evenly in small clumps (Pl. 10, fig. 9), and there is a conspicuous oval nucleolus which stains red with Mallory's stain. The cell outlines are not distinct, but where one would expect the cell boundaries to be there are accumulations of large clear vacuoles (Pl. 10, fig. 9), undoubtedly the secretory product of these cells. In the peripheral cells of this group the cytoplasm is not very vacuolated, the vacuoles being very markedly intercellular: but more centrally and towards the anterior end the whole of the cytoplasm of the cells is full of small vacuoles while the larger vacuoles lie in between the cells. In this region the proximal group is seen to be attached to the distal group of gland-cells (Pls. 9 and 10, figs. 3 and 9).

The proximal group is supplied by a small branch of the nerve to antenna 1 which comes off very near to the brain. There is no efferent duct from the proximal group as described by Claus.

The Distal Group.—The distal glands (Text-fig. 1) on each side consist of five cells, four gland-cells and a duct-cell. The gland-cells are arranged in two pairs situated anteriorly and posteriorly, connected with each other—the hinder pair embracing the duct-cell.

The anterior pair of cells are in direct connexion with the posterior side of the nerve to the first antenna at a point a little further from the brain than the branch to the proximal group (Pls. 9 and 10, figs. 2 and 13), and there is a conspicuous group of nerve-cells in the nerve in this region (Pl. 9, fig. 2). Laterally, as stated above, these cells are connected with the proximal group, and at this point the vacuolated cytoplasm of the proximal gland-cells is seen to be continuous with that of the distal gland-cells, the vacuoles passing freely from one group to the other (Pl. 10, fig. 9). The peripheral cytoplasm, except at this point of juncture, is denser than that in the interior of the cells, and is free from vacuoles of secretion (Pl. 10, fig. 9). There is no distinct division between these two cells, but in between the two nuclei there is a confused mass of vacuoles.

Centrally these vacuoles coalesce and form an irregularly flat, ill-defined reservoir (Pl. 10, fig. 9). The vacuoles are not very transparent, and in passing from the proximal glands to these two cells of the distal glands one can see the vacuoles becoming more opaque.

The nuclei are not cup-shaped as Cunnington (**5**) stated to be the case generally with the nuclei of the distal glands, but are roughly spheroidal (Pl. 10, fig. 9). Their diameter is not usually so great as the length of the largest nuclei in the proximal group, but there is probably not much difference between the volumes of these nuclei. There are larger clumps of chromatin in the nuclei than in those of the proximal group, and also the nucleoli, which stain red with Mallory's stain, are about twice as large. But there is also a diffuse scattering of chromatin all through the nucleus which gives it a much darker appearance in a stained preparation.

These two anterior cells of the distal group are connected by an attenuated process with the two posterior gland-cells (Text-fig. 1 ; Pl. 10, fig. 13). The reservoir in the anterior pair is not continuous as a duct through this drawn-out connexion, but vacuoles are to be seen here, so that presumably the secretion can pass from the anterior to the posterior pair of cells. This connexion is always attached to the dilatatores oesophagi (Pl 9, fig. 4), and its middle point is a little posterior to the labral nerve loop (Pls. 9 and 10, figs. 4 and 13).

The nuclei of the posterior pair of gland-cells are cup-shaped, as Cunnington states. Most of the nucleus forms a thin lamella but there is usually a swelling in the region of the nucleolus (Pl. 10, fig. 12). This is large and usually flat and shows the same staining reactions as the nucleoli of the other gland-cells. The chromatin is gathered together in clumps as shown in Pl. 10, fig. 10, but a more irregular clumping as shown in Pl. 10, fig. 12, is more characteristic.

The cytoplasm is pervaded with vacuoles of secretion which are opaque to varying degrees, and these are very conspicuous in the hemispherical recesses formed by the nuclei. As before,

there is no distinct division between these two cells, but the nuclei are placed with their concave sides facing towards each other and in between the two is a very conspicuous and clearly-defined reservoir (Pl. 10, figs. 10 and 12). This is apparently formed of a flat plate of transparent coalescing vacuoles of the secretion produced by the gland-cells.

Neither of these cells possesses an efferent duct as figured by Cunnington, but posteriorly they embrace a separate duct-cell (Pls. 9 and 10, figs. 6, 11, and 12). This cell has the form of a tube opening to the exterior at its posterior end and anteriorly opening into the reservoir of secretion. The lumen of this tube is often flat (Pl. 10, fig. 11) especially at its posterior end. The nucleus of this duct-cell stains very lightly and is small compared with that of a gland-cell, although it is slightly larger than that of a nerve- or muscle-cell. The cytoplasm stains very lightly and is not vacuolated.

In sublimate material there is in the secretion reservoir a granular coagulum which stains faintly blue with Mallory's stain, while in the lumen of the duct-cell it stains red. Presumably the cytoplasm of the duct-cell alters the constitution of the secretion in some way, so that its staining reaction when fixed is changed. A section through the duct-cell at its anterior part shows the secretion in contact with the walls of the tube staining red, while that more centrally placed, which has not yet been acted upon by the duct-cell, still stains blue. The external apertures of the duct-cells form two small slits on the side of the labrum near its tip (Pl. 9, fig. 7) where the latter is compressed laterally. They are situated a little towards the dorsal surface of the labrum and are ventral to about mid-way between the mandibles and maxillae.

In other Daphnids studied it was not found possible to obtain preparations sufficiently well fixed on which to base critical considerations, but it is evident that the same ground-plan underlay all the cases studied. In Chirocephalus, however, the results obtained are very good and agreed comparatively well with Claus's (4) figure for Branchipus. The proximal group is very scattered and ill defined. Its cells do not all line

the chitinous cuticle, but, however, they are connected with the gland-cells of the distal group and loosely fill the anterior part of the large labrum. The distal group is represented by three pairs of gland-cells—two placed laterally and one medially—slightly nearer the tip of the labrum. The nuclei of these cells are very large but not cup-shaped. In each pair of cells is a secretion reservoir which opens into the lumen of a very conspicuous duct-cell just as in Simocephalus vetulus.

On the Manner of Feeding.

Simocephalus vetulus feeds on small particles and planktonic organisms contained in a current of water which it maintains over its mouth appendages. In observing the animal it is usually on its back as figured in Text-fig. 1, but in describing the method of feeding, to avoid confusion, the animal will be assumed to be dorsal side uppermost.

The valves of the carapace form an incomplete tube about the posterior part of the animal, this tube being effectively completed by the hairs along the ventral edges of the carapace (Text-fig. 1). Posteriorly the tube is open to the exterior and anteriorly it expands at each side of the labrum into the bays from which arise the second antennae. Further, this tube is incompletely divided into a dorso-lateral chamber, which includes the brood-pouch and in which are the branchiae, and a median ventral food passage. The latter is bounded dorsally by a well-marked food groove (Pl. 9, figs. 6, 7, and 8) which runs along the ventral side of the trunk. Ventral to it are the hairs along the edges of the carapace while laterally are the trunk limbs. The current of water carrying the food passes in at the bases of the second antenna, and so passes close to the first antenna on which are situated, according to Scourfield (17), the supposed olfactory organs, and passes out at the postero-ventral angle of the carapace in the neighbourhood of the anus.

The appendages chiefly responsible for maintaining the food-stream are the first, third, and fourth trunk-limbs. Calman (2) states that the third and fourth pairs of trunk-limbs 'are

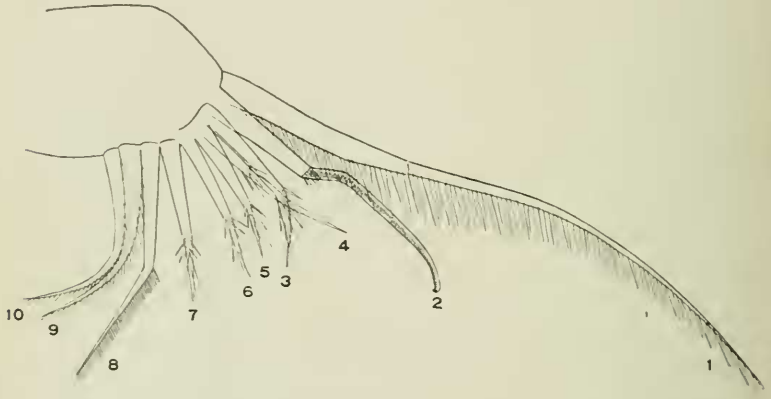
characterized by the development of the proximal endite with its comb-like row of setae '. These endites are placed almost vertically with their setae pointing upwards into the food groove. They diverge slightly from behind forwards and in passing upwards towards the trunk they slope inwards. They move in and out laterally. From the fact that they are nearest together at their posterior end the outward movement sucks in the water from before backwards. Since also they are not placed vertically but are slightly further apart at their proximal end than they are at the end of the comb of setae in the food groove, the outward movement, in all probability, causes a small backwash in a forward direction in the food groove.

Although the food current is produced mainly by the third and fourth trunk-limbs the first also plays an important part. The shape and arrangement of the first trunk-limbs can best be seen from Text-fig. 1. Its setae form a curved shield over, that is, ventral to, the second trunk-limb. In its normal movement it synchronizes with the other trunk-limbs but is not in the same phase. It commences its backward stroke just after the other limbs begin to beat outwards. The outer part of the limb moves in an arc of a circle with the tip of the labrum as centre (Text-fig. 1) so that those setae which lie against the side of the labrum scarcely move at all. The two limbs together thus form a funnel-like entrance to the food passage down the centre of which projects the labrum. The reason for the retarded lateral movement of this pair of limbs is not at all certain, but in all probability it is to secure a passage of water over the branchiae.

The second trunk-limbs are peculiar in possessing a large and specialized processus maxillaris or gnathobase. Their exopodites or outer branches lie over, that is ventral to, the succeeding limbs, and their function is probably merely to assist by their oar-like movements in maintaining the food-stream. The gnathobases point inwards and are beset with setae which point inwards and almost meet in the middle line. On each gnathobase there are ten setae. The posterior seven point backwards while the anterior three point forwards. They

are numbered in Text-fig. 2 from behind forwards. No. 1 is very long, reaching back to the hind end of the body, and is beset with long hairs. No. 2 is much shorter and ends in a small hook, and possesses a comb-like row of minute closely-set hairs over a little more than half its length on one side. Nos. 3, 5, 6, and 7 really form a series. They are short stout setae ending in a brush-like tuft of hairs. No. 4 differs slightly from them in being shorter but terminating in a long thick

Text-fig. 2.



Gnathobase of second trunk-limb of Simocephalus vetulus (for explanation see text).

hair projecting beyond the rest. Lilljeborg (**13**) does not figure this difference. Nos. 8, 9, and 10 have the form of forwardly projecting combs. No. 8 is usually bent at an angle at about its middle point, while Nos. 9 and 10 are curved. The hairs on No. 8, which only occur on its distal half, are very fine and regular, and are about twice as closely set as those on Nos. 9 and 10, which occur along the whole length of the setae. During the movement of the second trunk-limb outwards and forward the gnathobase also moves upwards so that its three anterior setae comb the side of the tip of the labrum. When the limb is in its most forward position these three setae have passed across the labrum on to the maxillae.

Each maxilla is armed with three setae which do not point dorsally as figured by Cunnington (**5**), but point forwards along

the food groove (Pl. 10, fig. 13, and Pl. 9 figs. 5 to 8). Each seta is beset with a double row of hairs on its inner side. During the movement of the maxillae the setae move backwards and forwards and in their forward movement move inwards, so that the hairs of the opposite setae meet in the food groove.

At the hinder margins of the biting surfaces of the mandibles there are blunt spines (Pls. 9 and 10, figs. 6 and 13), while the anterior parts are scored with vertical serrated ridges. The two mandibles, which are never symmetrically apposed to one another, appear to work like two cog-wheels fitting into one another and thus crush the food and at the same time force it forwards into the beginning of the oesophagus, up which it rapidly passes by peristalsis.

The mechanism of the method of feeding is as follows: food particles in the food-stream, drawn in by the action of the united movements of the trunk-limbs, are diverted towards the median groove along the side of the labrum, by the first trunk-limbs. At the tip of the labrum they are caught by the anterior setae of the gnathobases of the second trunk-limbs and brushed dorsally into the food groove above the tip of the labrum and between the maxillae. The brush-like setae of the gnathobase are in all probability the main agents in bringing this about. The more anteriorly-placed comb-like setae which brush the side of the labrum also assist in collecting the food on to the maxillae, but their chief function seems to be to brush the secretion of the labral glands on to the food as it collects between the maxillae. Hardy and MacDougall (8) state that when the food is swallowed it consists of particles—'which are glued together by some sticky substance'. It is suggested that this sticky substance is the secretion of the labral glands. The food which collects as a bolus between the two maxillae is now and again pushed forwards by the movements of the appendages on to the mandibles. Pl. 10, fig. 13, shows how the hairs on the setae of the maxillae point forwards, and Pl. 9, figs. 6, 7, and 8, show how the hairs of the adjacent setae fit together and so make an admirable broom for sweeping a bolus forwards on to the mandibles. A movement of the maxillae

is always followed immediately by a movement of the mandibles, but the latter rotate many times without any movement of the maxillae, so that probably the maxillae push forwards a large bolus on to the mandibles and these gradually pass it into the oesaphagus.

Hardy and MacDougall (**8**), referring to Daphnia, which is no doubt essentially similar in its feeding to Simocephalus vetulus, state that food particles are carried over the mouth by a current of water and ' many of them adhere to the sticky surfaces of the mouth appendages ', and that these adherent particles are formed into a bolus by the movements of the appendages. To observe the method of feeding these workers fed the Daphnids on milk, yolk of egg, and carmine. When the animals are fed on any of these substances they always become dirty, the particles adhering all over their bodies. With the former two substances they become greasy and break through and adhere to the surface of the water. It is thought that this is merely due to the presence of an abnormally large quantity of food. In the normal animal, feeding on its normal food, no particles are to be seen adherent to the appendages. If the animal is at all moribund it soon becomes covered with adherent particles.

If the animal be fed on milk—a drop of milk is carefully placed at the bottom of a watch-glass containing the water in which the water-fleas are swimming—the regular movement of the appendages is often stopped while the setae of the first trunk-limb are combed over the lateral surface of the labrum to remove any milk adhering to it. Also by this method of feeding a large amount of fatty drops collect in the food groove posterior to the maxillae. These are in all probability drawn there by the backwash previously mentioned that must pass out along this groove. When this accumulation of food becomes too great the labrum is raised by its levator muscle—which runs from the base of the labrum to the covering of the brain—the trunk is flexed forwards, and, with the caudal furca, the accumulation is lifted out of the food groove and, by the extension of the body, removed to the exterior.

On the Function of the Labral Glands.

Some preliminary experiments of staining Simocephalus intra vitam suggested that further investigations might elucidate the functions of the labral glands. The experiments which were accordingly carried out did not prove of much use in the direction expected, but were interesting and will be described here.

Fischel (**6**) describes experiments on intra vitam staining using, among other stains, alizarin, neutral red, Bismarck brown, Nile blue sulphate and hydrochloride. In repeating his experiments using the stains named, the only stains with which successful results were obtained were neutral red and Bismarck brown. It may be mentioned that these two stains were Grübler's chemicals while the others were not.

In Fischel's figure of Daphnia magna stained intra vitam with neutral red, there are figured two large red patches in that region where the labrum should be drawn which probably represent the labral glands. He states that these glands are always to be found faintly stained in animals stained intra vitam with neutral red. In adult Simocephalus vetulus the most conspicuously-stained organs in such animals are the labral glands and the body which Fischel describes as a gland of unknown nature, which has since been shown by Langhans (**10**) to be the end-sac of the shell gland, and both these stain intensely. In the labral glands both proximal and distal groups stain, but the duct-cell remains unstained. The connexion between the anterior and posterior pairs of cells of the distal group appears very distinctly, and was at first thought to be a distinct duct. In the gland-cells there appear accumulations of an intensely staining material—these accumulations being often as large as the nuclei of the cells. The reservoir of secretion which can be seen in the living animal remains unstained.

Fischel maintains that the staining with neutral red is not due to the staining of passive metabolic products but to the staining of preformed elements in the protoplasm. In support

of this he states: 'ist einmal die Granularfärbung eingetreten, so bleibt sie auch konstant, das Bild derselben ändert sich in keiner Weise, wie lange auch die Tiere beobachtet werden mögen. Und was ebenso wichtig ist, färbt man eine grössere Anzahl von Tieren, so weisen Zellen der gleichen Art stets auch die gleiche Granulierungsart auf'. In the experiments on Simocephalus vetulus no such constancy was observed in the labral glands. While these remained stained they did not continually present the same appearance; moreover, not only did the glands of different individuals stain differently, but the glands of the different sides of the same individual stained differently, which is what one would expect from the mobile, vacuolated nature of the protoplasm constituting the labral glands. However, quite apart from this case, this constancy in the appearance of a cell stained intra vitam with neutral red does not agree with the fact that by such staining methods the mitochrondria are stained (Gatenby (7)). Lewis and Lewis (11) have shown that not only do mitochondria continually change their shape but also are continually shifting their position.

If specimens are fixed in sublimate after staining intra vitam with neutral red and dehydrated rapidly some of the stain remains in the specimen. If they are now embedded and sectioned, on mounting the ribbon the stain can be seen in patches in the labral gland, and the position and shape of these can be drawn with reference to the contour of the glands. If now the wax is removed and the sections brought down to water the remaining stain is washed out. Staining now with an aqueous solution of thionin there appear dark bodies in the section staining an intense violet, almost black, and these patches agree with those stained by the neutral red. In sections of the animals which have not been stained with neutral red but which had been similarly fixed and stained in thionin, these very conspicuous dark bodies do not occur, and it seems safe to assume that they are formed by the action of the neutral red on the animal.

Weak solutions of neutral red apparently always have a harm-

ful effect on Simocephalus vetulus. No individual of Simocephalus exspinosus was found to survive a weak solution longer than twelve hours. In Simocephalus vetulus the movements of the limbs is always retarded when the animals have been in such a solution for about twelve hours. Advantage was taken of this fact to study the movements of the limbs during feeding. Usually, even if the stained individuals are removed to pure water, they survive only a few days. Sometimes, however, with young individuals they survive and completely lose all effects of the stain. No adults have been obtained to survive long the effects of the stain, but among these adults the stain often shows signs of disappearing and yet the labral glands always remain as conspicuously stained as at first. It was thought from these results that the labral glands might be partly the agents causing the disappearance of the neutral red. However, in the well-known experiment of feeding Daphnids on carmine, while the end-sac of the shell gland is stained by the carmine there is never any trace in the labral glands. This experiment was also repeated with neutral red, Bismarck brown, Nile blue sulphate and hydrochloride, using a filtered mixture of the stain with milk to feed, but there was no indication as to where the stain was excreted.

Apparently with neutral red and Bismarck brown the staining effect is not produced through the gut but the stain acts directly through the cuticle. Thus young embryos in the brood-pouch stain just as markedly as their parent. Both these stains show a great affinity for yolk. Individuals with nearly fully-developed embryos in the brood-pouch were stained in neutral red for twenty-four hours. Those individuals were then selected which had given birth to their brood, but had not yet laid their next batch of eggs, and these had deeply-stained ovaries. These were returned to fresh water. The eggs which were subsequently laid were stained deep red. As these developed the stain was seen to be confined chiefly to the yolk. In most cases the adults died before giving birth to the young but in a few cases the young were born, but the adults never

survived the succeeding ecdysis. The young in these cases showed stain chiefly in the tips of the first and second antennae and in the ' Haftorgan '. By the third instar all traces of the stain had disappeared.

These experiments show a similarity to those of Sitowski (18) on Tineola biselliela. This worker fed these caterpillars on food stained with Sudan red, and their fat became stained red, giving them a red appearance. The eggs laid were also stained red while the animals hatching from them showed signs of a slight red coloration.

They are also most probably similar to a certain experiment of Agar (1) on Simocephalus vetulus. In Agar's experiment he fed the Daphnids on a food which produced in them a curious abnormality, which consisted in a change from the normal, of the curvature of the valves of the carapace. On removing the abnormal individuals to normal conditions the abnormality disappeared in a few generations, and up to this point the result is analogous to Sitowski's results and to the experiment recorded here. However, Agar states that not only did the abnormality disappear, but in the third generation of the offspring there was a ' very decided reaction '—the valves of the carapace not only came back to their normal position but overshot the mark and became more curved in the opposite direction. This is stated to be due to the overproduction of an anti-body antagonistic in its effects to the substance causing the abnormality. This occurrence of a reaction was supported by a table of ratios representing the transmission of the abnormality, and about this table Agar says that, by itself, ' it cannot be said to give unequivocal evidence, especially when the high degree of inaccuracy in the original measurements is considered ', but that this table bore a ' striking resemblance ' to a second table representing the transmission of another abnormality which was based on much more accurate measurements and on a much greater number of individuals. But, even supposing that this latter table accurately represents the course of the second experiment, the value of the resemblance

between the two tables as a basis on which to postulate similarity between the two sets of experiments, of which the tables are representative, depends solely on the accuracy of the first table. While it is admitted that the second table is no doubt comparatively accurate, it must be emphasized that in the first table referring to the abnormal curvature of the carapace, the presence of a reaction in F 3 generation was indicated by an increase among only forty-seven individuals, of 6 per cent. over a normal ratio—and in measuring this ratio an error could be made of as much as 20 per cent.—the average error according to a table quoted by Agar to show this inaccuracy is roughly 10 per cent. It appears very uncertain, on such data, to make the definite statement that there was a ' very decided reaction '. The repetition of these results of Agar was abandoned because, in the individuals used, the inaccuracy of the measurement of the ratio which indicated the extent of the abnormality was even more marked than in those used by Agar.

It may be mentioned here that Agar merely stated that the food producing the abnormality was a ' culture of protophyta grown in a mixture of cowdung, soot, and water '. It was found that the abnormality can be produced by feeding a culture containing no other protozoon than a species of Chlamydomonas. Also, contrary to Agar's finding, it was not found possible to produce the abnormality in Simocephalus exspinosus.

In the experiments recorded here there seems no evidence as to how the neutral red disappeared. As Agar suggests for cases of parallel induction, it may have disappeared by mere dilution caused by the increase in the bulk of the protoplasm without a corresponding increase in the amount of the stain. Partly the stained matter may be oxidized or changed in some way into a colourless material which may or may not be excreted ultimately.

These experiments on intra vitam staining were carried out before the mechanism of feeding was closely studied. The latter investigation made it obvious that, as already stated,

the food is entangled in some substance before it reaches the mouth, and from the disposition of the appendages and their method of working it seems most probable that this substance is produced by the labral glands. This brings Simocephalus vetulus into the same category of feeders as those Gastropoda, Pelecypoda, Protochordata, and Branchiopoda whose method of feeding is described by Orton (**14**), in which the prehension of the food is brought about by the secretion of some food-entangling substance. The nature of this substance in Simocephalus is, however, peculiar. Sections of the glands were stained according to the method recently described by Keilin (**9**) using thionin as a metachromatic stain for mucin. The nuclei of the gland-cells stained blue while the cytoplasm was purplish, as would occur in a mucous gland, but the secretion filling the reservoir not only did not stain red, as it would do if it contained mucin, but showed a pale-blue tint. Bismarck brown also left the contents of the reservoir unstained. If, then, the metachromasy of thionin is used as a definite method for the detection of mucin, the labral glands of Simocephalus vetulus must not be described as mucous glands.

From the quotation from Schödler's work on Acanthocercus at the beginning of this paper it will be seen that he suggested that the labral glands were possibly salivary glands. Claus does not discuss their function but merely states: ' Die grossen Zellen der Oberlippe . . . betrachte ich als Lippendrüsen '. Cunnington, discussing the physiological significance of the secretion from the labral glands, states that the fact that the secretion flows out in front of the mouth suggests that the gland is a salivary gland.

The term ' salivary gland ', derived as it is from vertebrate and more especially human anatomy, is now unfortunately used in a variety of senses in the different groups of animals. In some groups a certain physiological sense is implied, while in others the term is used only in a topographical sense. Among the Arthropoda, it is not possible to find, from the physiological sense, a character common to all the secretions of their salivary glands, while from a morphological standpoint,

owing to the very uncertain homologies of the various mouth parts in the different classes, it is not advisable to base a definition of salivary gland in this group on such considerations. Hence the term salivary gland should not be extended still further to include the labral glands of Simocephalus vetulus.

South Kensington.
August 1921.

Summary.

1. The labral glands consist of a proximal and a distal group of gland-cells.

2. The proximal group consists on each side of about twenty cells. The cells possess large flat nuclei and their secretion collects as intercellular vacuoles.

3. The distal glands which are in connexion with the proximal groups consist on each side of five cells—four gland-cells and a duct-cell. The anterior pair of gland-cells possess large spheroidal nuclei between which is an ill-defined reservoir of secretion. The posterior pair have cup-shaped nuclei between which is a very definite reservoir of secretion.

4. The duct-cell is in the form of a hollow tube, one end opening to the exterior near the tip of the labrum and the other end opening into the reservoir of secretion between the nuclei of the posterior pair of distal gland-cells. The duct-cells act as ducts for the whole of the labral glands, the secretion passing as vacuoles from cell to cell.

5. The duct-cell alters the reaction of the secretion before passing it to the exterior.

6. Food particles carried in the stream which is maintained by the trunk-limbs through the carapace are abstracted by the gnathobases of the second trunk-limbs.

7. There are ten setae on the gnathobase of the second trunk-limb, the anterior three of which are comb-like and brush the secretion of the labral glands on to the food particles as they collect between the maxillae.

8. The setae of the maxillae are directed anteriorly, and by their action pass the food on to the mandibles at the entrance of the oesophagus.

9. The labral glands stain very markedly intra vitam with neutral red and Bismarck brown. There is no evidence that this effect is due to the staining of the preformed structures in the protoplasm.

10. Females stained intra vitam with neutral red, when removed to fresh water will lay red eggs from which young will hatch which are also stained. The stain disappears from these during growth.

11. Agar's experiments on the transmission of an abnormality produced by a certain food are criticized. This abnormality can be produced by feeding Simocephalus vetulus with Chlamydomonas.

12. The secretion of the gland contains no mucin.

EXPLANATION OF PLATES 9 AND 10.

Description of Figures.

All figures are from Camera lucida drawings. Figs. 1–8 were drawn using a Zeiss D. objective and are at a magnification of 222 diameters. Figs. 9–12 were made with a Zeiss apochromatic N.A. 1.4, 2 mm. objective and compensating ocular 8. The magnification is about 860.

List of Abbreviations.

a.l.g. external opening of duct of labral gland; *br*, brain; *c.c.* circumoesophageal commissure; *d.c.* duct-cell; *d*, duct of labral gland; *d.o.* dilatores oesophagi; *e.c.* epidermal cell; *f.g.* food groove; *ga* 1, ganglion of Antennarius 1; *g.pg.* group of nerve-cells in Antennarius 1 at root of branch to proximal group; *l*, labrum; *l.d.c.* lumen of duct-cell; *l.g.* lateral groove dividing labrum from dorsal part of head; *l.l.* labral nerve-loop; *md*, mandible; *mg*, mid-gut; *mx*, maxillae; *n.a.* 1. Antennarius 1; *n.a.* 2′. Antennarius 2 major; *n.a.* 2″. Antennarius 2 minor; *n.md.* mandible nerve; *n.mx.* nerve to maxilla; *n.n.e.* nerve to nauplius eye; *n.pg.* nerve to proximal group of gland-cells; *nl*, nucleolus; *nu.p.g.* nucleus of proximal

gland-cell; *nu.a.d.* nucleus of cell of anterior pair of distal gland-cells; *nu.p.d.* nucleus of cell of posterior pair of distal gland-cells; *nu.d.c.* nucleus of duct-cell; *oe*, oesophagus; *o.g.* olfactory ganglion; *p.c.* peripheral layer of cytoplasm of anterior pair of distal gland-cells; *r.* reservoir of secretion; *s.m.* setae of maxilla; *v.* vacuoles of secretion.

Figs. 1–8 form a series of transverse sections through the labrum and adjacent parts of an adult specimen of Simocephalus vetulus. The position of these sections is indicated in fig. 13 by the series of vertical lines numbered 1–8 at their upper ends. Figs. 9, 10, and 11, are drawings at a higher magnification of parts of the same sections that are represented in figs. 3, 5, and 6 respectively. The orientation of figs. 9–11 with respect to the plate is the same as it is in figs. 3, 5, and 6. In figs. 1–8 and in fig. 13 the proximal glands are shaded thus and the distal glands are shaded thus .

Fig. 1.—Section cutting the most anterior part of the proximal gland at the level of the nerve to the first antenna.

Fig. 2.—Section through anterior end of the distal gland. This section passes through the nerve to the proximal gland.

Fig. 3.—Section through the connexion between the proximal and distal groups of gland-cells.

Fig. 4.—Section through the attenuated connexion between the anterior and posterior pairs of gland-cells of the distal gland. This section passes through the commencement of one of the mandibles and includes the labral nerve-loop.

Fig. 5.—Section through the posterior pair of cells of the distal gland. This section includes the tips of the hairs on the setae of the maxillae.

Fig. 6.—Section through the duct-cell.

Fig. 7.—Section through the external opening of the duct of the labral gland.

Fig. 8.—Section through the maxillae and the tip of the labrum.

Fig. 9.—See fig. 3.

Fig. 10.—See fig. 5.

Fig. 11.—See fig. 6.

Fig. 12.—Horizontal section, the position of which is indicated in fig. 13. It passes through the aperture of the duct of the labral gland.

Fig. 13.—Somewhat diagrammatic figure of a side view of Simocephalus vetulus. Of the appendages behind the antennae only the right mandible and maxilla are represented.

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